SPERMATOGENESIS OF THE DRAGON-FLY SYMPETRUM SEMICINCTUM (SAY) WITH REMARKS UPON LIBELLULA BASALIS

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CONTENTS.

Observations upon the changes which occur during the ripening of the male sex products in *Sympetrum semicinctum* (Say) are described in this paper. The account, beginning with the last multiplication division of the spermatogonia, traces especially the behavior of the chromatin through the growth and maturation periods up to and including the formation of the spermatozoa.

The sex-chromosome, a round, univalent body, appears at the beginning of the growth period and by its different staining capacity can be traced as a definite body throughout the changes

which take place in the nucleus, from the pre-synaptic period up to the formation of the spermatid, where it loses its identity and combines with the chromatin derived from the autosomes. The exact behavior of the autosomes is difficult to make out in all details. They apparently unite by a side to side union, or parasynapsis, and later separate along the line of union to form rings which condense into quadripartite elements.

For comparison, another species of dragon-fly, *Libellula basalis*, was also studied.

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I. Nymphs.

Several hundred nymphs of two species, *Sympetrum semicinctum* and *Libellula basalis*, were collected in the spring and fall of 1912. Most of those obtained in the spring were found in the ooze of the marsh before they had come up for transformation. In the fall, the majority were found clinging to reeds and roots of grasses along the bank of a little stream on the University farm. The most prevalent form was *Sympetrum semicinctum* (Say). See Fig. 1, for a drawing of a nymph. A large number of nymphs secured early in the spring, 8 to 10 mm. long, must have belonged to the brood of the preceding year. Other nymphs taken in the spring measured 17 or 18 mm. Some of these had well-developed testes and were about ready to transform, while others had no visible gonads. Of the nymphs obtained in the fall, many were small and had evidently hatched from eggs laid during July or August, while most of the large ones had reproductive organs just developing. These facts indicate that the larval period extends over more than one year.

II. Testes.

The testes of the nymphs appear as two white filaments, one on either side of the digestive tract, extending almost the entire length of the abdomen. They are not composed of lobules like

the testes of certain other insects, but consist of globular cysts arranged one or two layers deep around a central duct which runs through the organ in more or less of a zig-zag course. Each testis is of nearly uniform size along its entire length, tapering at the posterior end where the vas deferens emerges. The epithelium covering it is thin and hidden by a layer of fatty tissue which contains tracheæ. The central duct has a thick epithelial wall and each cyst, surrounded by a thin layer of epithelium, apparently opens into the central lumen by a separate duct. All the developing stages of the spermatozoa may in favorable preparations be found in one cross-section. The cysts seem to have no definite arrangement in the tubule according to the age of the products. One containing primary spermatocytes may lie between two containing mature spermatozoa. As a rule all the cells in each cyst are at the same stage of development, although two or three primary spermatocyte divisions may occur occasionally in cysts containing older products. Where the cell is passing rapidly through the later prophase stages which precede the first maturation division, cysts containing two closely successive stages occur. In the older cysts which contain spermatozoa, there is a shrinkage in size and the cysts become separated by large spaces. Fig. 2, a cross-section of a whole testis, shows the central duct with its thick epithelial wall; the cysts containing products at different stages of development; and the fatty layer containing the tracheae and covering the outer epithelium of the testes. In some testes degeneration had taken place in a few cysts containing spermatogonia. Fig. 3, a cyst in which degeneration had taken place, shows how the chromatin condenses into a solid mass in the nucleus of each spermatogonium.

In the female larva, the ovaries lie in the anterior end of the abdomen, dorsal to the digestive tract and are close together anteriorly, while posteriorly they separate forming an inverted V.

III. Method of Fixing and Staining.

Injection of the living larva with the killing fluid was found to be the best method of killing and fixing the gonads. A hypo-

dermic syringe with a small needle was used. The dorsal part of the abdomen was then cut off and the nymph placed in a dissecting pan of water. The testes were removed as quickly as possible to a vial containing the fixing fluid used for injection. Where Bouin's fluid was used for killing, the dissection was made in 70 per cent. alcohol instead of water. The best results for working out the different stages, especially those of the growth period, were derived from fixation in Bouin's fluid followed by Heidenheim's iron-hæmatoxylin with eosin or acid fuchsin as counter stains. Some excellent preparations were obtained by staining with an aqueous solution of safranin for two minutes, followed by lichtgrün. Good results also followed the use of a saturated aqueous solution of Gentian violet and orange G after fixation with Flemming's solution.

For quick observations upon fresh material aceto-carmine was used. Cells stained with this swell slightly but cytological details such as chromosomes, spindle, centrosome and spireme are brought out clearly. Satisfactory counts of polar views and camera-lucida drawings could easily be made. The details obtained in these entire cells could be used as a check in examining sections.

Testes were also teased and mounted unstained upon a slide in Ringer's and in physiological salt solutions. Normal saline caused plasmolysis after a short interval, but tissue placed in Ringer's solution made up with a .5 per cent. normal salt instead of .7 per cent. remained normal for several hours before signs of disintegration began. The chromosomes in both *Sympetrum* and *Libellula* could be seen in the fresh material, as they have a refractive power which differs from that of the cytoplasm. The chromosomes upon the spindles both in the metaphase and anaphase could be distinguished as separate bodies in the primary spermatocyte division. In growth stages a spireme was visible while in the spermatogonia the chromatin nucleolus was evident. This proves that the details of the cell, as revealed in preserved materials, are reasonably faithful presentations of the conditions which really prevail in the living cell. Complete cell division was not observed. Cells teased apart remain connected by long protoplasmic threads. The fact that the chromosomes

can be separately distinguished opens up the possibility of experimentation upon cell division when a solution isotonic with the body fluid of the dragon-fly nymphs can be determined.

IV. Spermatogenesis of Sympetrum semicinctum (Say).

(*a*) *The Spermatogonial Period.*

The length of larval life in this form is unknown and it probably varies with temperature and food supply. Nymphs obtained early in May this year which measured 1 cm. in length were at least one year old for the adults had not begun to emerge. These nymphs possessed no visible gonads. In the youngest larvae bearing reproductive organs, it was exceedingly difficult to find spermatogonial divisions. Until the life cycle is known no explanation can be offered for this, but it may be that the gonads develop during the winter of the second year of larval life after the insects are in the mud at the bottom of the streams where it is hard to find them. The material of this particular species was difficult to work with on account of: (1) the small size of the cells and their closely crowded condition; (2) the considerable number of chromosomes; (3) the irregular arrangement of the cysts as to age, referred to under III.

The spermatogonial cells could be easily distinguished by their large nucleoli and their large-mesh nuclear network. The chromatin granules, sparsely scattered along the linin network in the center of the cell, were collected in small clumps close to the nuclear membrane. This arrangement of the chromatin gave to the nucleus a clear appearance. Frequently small chromatin bodies appeared in the network, but these were inconstant in number and apparently of no importance in the later development. The nucleolus appeared more often as composed of a clear ground substance, probably linin, in which masses of chromatin were imbedded. It sometimes looked like a solid chromatin mass. Lewis and Lewis ('15) found that in a living cell, the nucleolus was never a compact body, but was coarsely granular and large in proportion to the nucleus. The degree of contraction of the ground substance inclosing the granules, depending upon the fixation of the material, would account for

the difference in appearance. The cytoplasm in some cells was clear and homogeneous, while in others it was alveolar and in most cases formed only a narrow sheath around the nucleus. Fig. 4 shows groups of spermatogonia with the structures just described, nucleolus, chromatin body, nuclear network and alveolar cytoplasm. Fig. 5 represents a spermatogonial cell from a smear preparation in which the chromatin granules appear as clumps near the nuclear membrane. Fig. 6 shows the 25 spermatogonial chromosomes in an aceto-carmine smear.

In the spermatogonial divisions, the undivided chromosomes could rarely be distinguished, as all the chromosomes in the metaphase usually blend into a black compact mass. In Fig. 7, a polar view of the metaphase before the last spermatogonial division, a few of the separate chromosomes can be seen. They are dumbbell-shaped, varying in size and are not as large as those found by McGill ('04), in *Anax junius* in the same stage. In some of my own preparations of *Anax junius* there were clear polar views in which 27 chromosomes could be counted. This verifies the corrected count of Lefevre and McGill ('08). In *Sympetrum semicinctum*, however, it was impossible to find many cells in which the chromosomes were sufficiently separated to permit of a satisfactory count. In many instances where partial counts were possible, more than twenty could be distinguished. Fig. 8 shows twenty-five plainly in a polar view of a telophase, and while the two cells in which twenty-five could be distinctly counted do not afford sufficient evidence from which to draw a conclusion, judging from the reduced number found in the primary and secondary divisions, the correct spermatogonial number should be twenty-five. The dense appearance of the polar view is due to the deeply staining proclivity of the threads which connect the chromosomes.

Figs. 9, 10, and 11 are telophase stages of the last spermatogonial division, showing approximately the amount of chromatin going to each cell. The telophase stages are more abundant than the metaphase ones in my material. A polar view of the telophase shows 25 chromosomes distinctly in one case and many show plainly 21 chromosomes and there are indications of others

which are not in focus. Figs. 12 and 13 show polar views of telophases of the last spermatogonial division.

The chromosomes soon fray out into indistinct masses as in Fig. 14. One, however, remains distinct and round in greatly destained preparations and may appear divided in some instances. Figs. 15 and 16 show the round chromosome as a single and as a double body. It is easy to distinguish this stage from the spermatogonium as the chromatin is thickly scattered throughout the cell in indistinct, granular masses. Some of these masses aggregate in the center or toward one side of the nucleus to form a nucleolus and the round body then is indistinguishable, obscured probably by the masses which formed the nucleolus (Fig. 17). There is then formed a vague, indefinite reticulum: the inconstant chromatic bodies mentioned as sometimes present in the spermatogonia are never present here. Wilson ('12), in stage A. in *Oncopeltus*, which is similar, says that in the early telophase, the sex chromosomes cannot be identified while in a little later stage, the sex chromosomes are elongated and the autosomes form a lightly staining, vague net-like structure in which individual chromosomes cannot be distinguished. Davis ('08) and McClung ('02*b*) also describe a like stage in the Orthoptera.

(*b*) *The Changes Occurring in the Growth Period up to and Including Formation of Crosses.*

As to exactly what takes place in synapsis it is hard to assert positively. There is present throughout this period a round, compact, deeply staining chromatin body which can be traced in every stage. This never loses its identity, though it may be obscured in some cells and from its subsequent behavior it can be identified as the sex-chromosome. It seems reasonable to suppose that it may be identical with the dense round body present at the end of the last multiplication period. But the disappearance of this body in the rest stage, although possibly only hidden under chromatin masses, breaks the continuity between the end of the last spermatogonial period and the growth period.

1. The nucleus has increased slightly in size and there is a corresponding increase in chromatin material, which is arranged into large, deeply staining, irregular masses. That the cell remains with the chromatin in the diffuse condition into which it passed at the end of the spermatogonial division for only a short time seems evident from the fact that few cells are found in that stage. Correspondingly large numbers contain the deeply staining masses. These masses are entirely different in appearance from those formed at the end of the multiplication period by the fraying out of the univalent, spermatogonial autosomes; they are larger, more irregular and stain more deeply. Their formation is not clear, the nucleolus separates into the chromatin particles which were loosely incorporated into it; the granules in the faintly staining network aggregate into clumps and the chromatin while increasing in bulk, develops a greater capacity for staining. Part of the process is a reversal of the behavior at the end of the spermatogonial division. These masses are so closely crowded that the number cannot be counted with certainty, but it is about equal to the diploid number. Most of them have ragged, irregular edges (Figs. 18, 19 and 20). One, marked X, stands out distinctly on account of its round, smooth outline and this later becomes the sex-chromosome. Toward the close of the stage, slender threads begin to extend out between the masses.

2. *Leptotene Stage.*—Each mass formed in the preceding stage ultimately becomes converted into a slender thread through the gradual outward migration of its component granules (Figs. 21, 22). The conditions correspond to that found by Montgomery ('11) in *Euchistus*, and by the Schriners ('06, *a* and *b*) in *Tomopteris*, *Spinax* and *Myxine*. The whole nucleus is now filled with fine, granular, much interlaced threads and it is impossible to determine their number. It may be that each mass only forms one thread, in which case there would be 24 threads which corresponds with the diploid number minus one.

The sex chromosome (Fig. 23) can be distinguished by its more compact make-up and more regular outline. It retains its shape and staining capacity, after the nucleus is filled with threads.

3. *Synapsis.*—In general, the leptotene threads are so scattered and tangled throughout the cell that it is impossible to be sure of their exact behavior. In occasional cells of a section where only a few threads remain, some threads can be seen to lie parallel to each other, while other threads are united at one end into V's (Fig. 24).

4. *Synizesis.*—All the threads drift to one side of the nucleus and what appears to be a continuous spireme is formed. If the leptotene threads paired previously side by side as they seem to do in synapsis, the spireme is formed by the end to end union of such paired threads. At this stage, the spireme thread is double (Fig. 27) and the two component threads are twisted and interlaced (Figs. 25 and 26). The spireme then becomes denser and thicker, possibly by the contraction of the loops which are closely drawn together near the side of the nucleus. The typical bouquet stage described by many investigators appears. There are in polar views through the loops 24 cut ends and as each loop is doubled back so that it would be cut through twice, the actual number of loops is twelve, which corresponds to the haploid number of autosomes.

The sex-chromosome is still a round, compact body and is seen best when it lies out near one of the larger loops. As a rule it is obscured in the loops near the nuclear wall.

The centrosome first appears at this stage and in some cells is divided. In others the two centrosomes are separated though there is no indication of the spindle. A large plasmosome sometimes appears in the cytoplasm as early as stage 1, but it is more common in this period. Figs. 28 and 29 show the spireme and Figs. 30 and 31 are polar views to show the cut ends. In Fig. 29 the sex-chromosome is shown among the loops of the spireme.

5. *Segmentation of the Spireme.*—The thickened spireme spreads out and breaks up into parts or segments, which are curved into horse-shoe shape loops. The segments are less than the diploid number, but it is not possible to count them accurately. A segment sometimes shows a split that is presumably the space between two leptotene threads which entered into its composition. That these are the same two threads which paired originally seems indicated in Fig. 32, where the

threads are twisted around each other into a chiasma. Jansen ('09) found that the threads of a tetrad became twisted and his figures resemble closely Figs. 32 and 33. The sex-chromosome is unaltered.

6. *The Condensation of the Segments into Crosses.*—The curved segments of stage five, now open up along the plane of the split to form loops and by becoming disunited at one end V's and U's occasionally occur. If the segments are extremely curved when the two threads split apart, rings bent in the form of eights are produced (Fig. 34). These do not correspond to the eights which some workers derive from such gemini because the threads are everywhere separated except at the two ends. An eight corresponding to one formed from a geminus sometimes results when the two component threads of a segment while remaining attached in the middle and at the ends, separate between the middle and the ends. Fig. 35 shows a true eight in the nucleus of a cell and the resemblance to the bent rings in Fig. 34 is apparent. The threads of each segment are granular and appear much like the leptotene threads of stage 2. In my preparations of *Anax junius* the chromomeres are plainly distinguishable in the threads (Fig. 36).

This stage is much like the prophase of the Orthopterans described by McClung ('14). Among the opened out loops, there are many modifications. The most striking one is the signet ring. If the two threads of a segment divide along the synaptic plane (a point which will be discussed more in detail later) a ring is formed which is composed of two autosomes united at both ends and pulled apart in the center. Now if a secondary split takes place in each thread and the two halves become separated at one of the synaptic ends, the signet ring type is explained. When viewed from the side such a ring appears as a loop with crossed ends (Figs. 37 and 38). By far the larger number of segments open out into true rings without this modification and this type was not found in every nucleus.

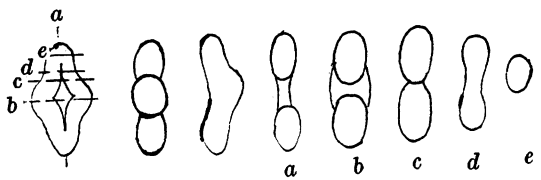
The two autosomes after opening out to form a ring remain connected at the ends. The granular threads condense into more compact ones and each apparently bends out in the middle in opposite directions until the connected ends which were far

apart are brought close together. At first the long axis of the loop coincided with the plane of union in synapsis, but with the bending of each autosome the long axis is reversed and lies at right angles to the original long axis. The ends where the chromosomes united do not bend, but remain extended to form side arms. A cross is thus produced by this process, the upper half composed of one univalent autosome and the lower half of another. When the signet ring condenses it assumes the same form as the others; all finally become condensed crosses showing a less dense area in the middle.

Figs. 39, 40, and 41 show cells containing a number of crosses, and in Figs. 40 and 41, the sex-chromosome which has retained its round compact form through all the stages of the growth period appears. In some prophases as many as nine different sizes of crosses which would form bivalent chromosomes of the primary spermatocyte could be counted. The largest bivalent autosome comes from a cross in which the secondary long axis is much extended and the united ends of the univalent autosomes bulge only slightly to form exceedingly short side arms (Fig. 41).

(*c*) *First Spermatocyte Division.*

The chromosomes, as last described, have become bivalent masses; with only an area less dense in the center to indicate the former central split. As just described, each bivalent has four projecting parts corresponding to the arms of a cross. Hence, if a bivalent is cut exactly in two, one half when viewed



TEXT-FIG. 1.

from the cut surface shows two ends connected by a cross piece below the level of the ends. It is difficult to give an adequate idea by a description but the text figure makes this clear by picturing the result in cross-section of different cuts through the bivalent.

Usually, in a polar view, the chromosomes appear bipartite or round because the cut does not exactly halve the bivalents. The sex-chromosome in such a stage of nuclear change is round and shows no evidence of a constriction. Figs. 42, 43 and 44 are polar views of the twelve bivalents and the single univalent sex-chromosome.

In *Sympetrum* there is usually an outer ring of seven bivalents and the sex-chromosome, surrounding five in the center. The chromosomes vary in size somewhat, although five are of nearly uniform size; one is larger and the others grade down gradually to the smallest which is smaller than the sex-chromosome. In some cases the latter may appear to one side, and then only four bivalents occur in the middle, one of the central bivalents apparently replacing the sex-chromosome in the outer ring. In a side view of this stage, all the autosomes are compact bivalents with four shortened arms and a central clearer area. The central bivalents can be seen below the others in an exact metaphase. The sex-chromosome can be easily distinguished from the bivalents by its rounded appearance and clear vesicle-like zone around it. All are connected with double spindle threads. The centrosomes are extremely large. The bivalents when dividing pull into two halves, presumably along the line of junction of the two ends of the autosomes which agrees with McClung's statement that separation between the parts of bivalent chromosomes is more likely to occur along spaces between whole chromosomes.

The bivalents do not seem to behave like tetrads when dividing. Occasionally they pull out into the form depicted in Fig. 48, but when the telophase is examined the autosomes show no split. If true tetrads were formed the telophase number of autosomes would be doubled or each autosome would be bipartite. Fig. 49 shows the sex-chromosome lagging behind the others and in Fig. 50 it is dividing after the others are well on their way to the poles. Figs. 51 and 52 are telophases of this division. Usually in later stages of division the sex-chromosome cannot be distinguished from the autosomes, but it occasionally stands out in a polar view of a telophase as in Fig. 53. In the telophase, the chromatin is massed at each pole, the cytoplasm

becomes constricted and the cells are divided. As in Fig. 51 a few spindle fibers may connect the two cells.

This is probably the true reduction division for the bivalents. For the sex-chromosomes which splits equally into two parts, this is an equational division.

(*d*) *The Second Spermatocyte Division.*

In the telophase of the primary division, the univalent autosomes are closely crowded together, but they soon separate and become scattered around the edge of the nucleus in preparation for the second division which closely follows the first. By actual measurement these cells are one half the size of the cells in the prophase of the preceding division. The univalent autosomes are small and compact. The second division occurs without any intermediate stages, the same autosomes which were in the preceding telophase become the metaphase autosomes. In polar view, they are round with no evidence of constriction and are arranged in the following order: a ring of 9, enclosing three, of which one is very small. This is nearly the same order as that occurring in the metaphase of the first division. The sex-chromosome, however, always lies outside the ring and is surrounded by its usual vesicle. This order prevailed in all cases counted although the size of the ring varied; the autosomes were spread apart more in some cells and in others they were collected into a smaller ring. In fact polar views of this stage (Figs. 54 and 55) appear much like the telophase of the first division. In Figs. 56 and 57 the sex-chromosome is not visible.

In side views of this stage, all the autosomes are dumbbell-shaped, with the two halves longer than broad. The sex-chromosome is at one side of the spindle and can be identified by its roundness, its vesicle and the lack of spindle fibers. During division, the sex-chromosome precedes the dividing autosomes and, undivided, goes to one pole. It may reach the pole before the autosomes start their division or it may be only a little in advance. In no spindle observed did it ever lag behind the others. Figs. 58, 59, 60, 61, 62 and 63 show spindles of the second division, with the sex-chromosome in various positions. In late telophases some of the spindle fibers may clump together

at the point where the transverse constriction of the cytoplasm takes place and a thickened thread results. This is shown in Fig. 64 which is a telophase. Fig. 65 is a drawing from an aceto-carmine smear in which the sex-chromosome stood out plainly at one pole. The centrosomes when present are much smaller than in the preceding division and in many cells they cannot be seen.

If the first division was reductional as the evidence seems to show, then theoretically this division must be equational. The sex-chromosome passed over undivided and this represents the reduction stage for it, as it divided equally in the first division. In *Libellula basalis* the sex-chromosome goes over undivided in the first division. Sutton (1900) found also that in *Brachystola* the sex-chromosome passed over undivided in the first division, so that as regards this element in different species, it is obvious that the place of reduction is not always the same.

(*e*) *Transformation of the Spermatid.*

At the end of the second maturation division each of the spermatids resulting from one secondary spermatocyte contains a mass of chromatin which never resolves itself into individual chromosomes. In many cases after the two daughter spermatids from one secondary spermatocyte are completely separated, the sex-chromosome stands out distinctly from the chromatin mass in one of the cells as indicated in Figs. 66 and 67. In most spermatids, the sex-chromosome is incorporated into the chromatin mass and there is no noticeable difference in the amount of chromatin in the two classes of spermatids. The chromatin becomes broken up into a number of irregular masses, three or more, connected by a faint network containing chromatin nodules at the intersections of the meshes (Figs. 68, 69, 70, and 71).

At this stage, the centrosome which lies close to the nuclear wall sends out a fine thread which is the axial filament of the tail. The cytoplasm at the end opposite the centrosomes elongates into a head spine which is free from granules and attains a considerable size (Figs. 72 and 73). Bütschli as far back as '71 described in the spermatozoa of *Agrion puellæ* a

head-spine which after increasing from .01 mm. to .45 mm. in length diminished with the ripening of the spermatozoa until it was only .0078–.009 mm. long. Fig. 74 is a drawing from a smear preparation showing the shape of the nucleus and the elongated head spine in the spermatid. The nucleus which at first is round, elongates and apparently enters the clear zone comprising the head-spine. The cytoplasm becomes a thin sheath around the nucleus which is extremely long and narrow. There is never a distinct middle piece, but the knob around the centrosome may be homologous to the middle piece found in other spermatozoa. In the ripe spermatozoa, this does not appear separated from the nucleus and the whole head stains as if composed of solid chromatin. The chromatin, however, is collected around the outer side of the nucleus, as cross-sections (Figs. 75 and 76) show a denser staining band of chromatin just beneath the nuclear membrane. The adult spermatozoa are motile and swim along with a twisting spiral motion. Figs. 77, 78, and 79 are drawings from aceto-carmine preparations in which the spiral twist of the adult spermatozoön is indicated.

There are apparently no external differences among the spermatozoa. In many spermatids drawn to scale no variation in size of head could be detected. Zeleny and Faust ('15) have found a dimorphism in the spermatozoa of *Æschna canadensis*, the ratio of the two 1.00 : 1.03. It is possible that using their method a dimorphic curve could be plotted for *Sympetrum*. However, the sperm on account of their method of locomotion would be fixed in varying twisted postures. When these are stained, their twisted condition is noticeable in only a few cases; most of the stained specimens appear as rods. This fact alone might cause such a discrepancy in size among the adult spermatozoa as to be mistaken for dimorphism even should the latter not occur. In *Sympetrum* from the mode of formation, one half the spermatozoa has more chromatin than the other half and this extra chromatin brought in by the sex-chromosome probably causes a physiological difference even if there is no visible dimorphism.

V. Some Notes on Libellula basalis.

The chromosomes of *Libellula basalis* in a general way undergo the same changes described for *Sympetrum*. The cells are larger; the diploid number of chromosomes is twenty-five, the reduced number thirteen. From the fact that certain cysts contain as few as eight large spermatogonia, while others contain large numbers of smaller ones, it is evident that several spermatogonial divisions occur. In several cysts in which an attempt was made to count the later spermatogonia, from 45 to 100 were present. The nucleus of a spermatogonium is usually eccentrically placed in the cell and part of the chromatin forms a large irregular nucleolus which is connected with chromatin nodules around the periphery of the nucleus by a few faintly staining threads. Fig. 80 shows such a cell from an aceto-carmine preparation, while Fig. 81 represents another from a section stained in iron-alum hematoxylin; Fig. 82 is a spermatogonium drawn under low power and is of interest since it was taken from a living cell in salt solution. In all the cells examined in this way, the protoplasm was drawn out into projections which resembled pseudopodia though no actual movement was observed.

In the prophase of the last spermatogonial division the chromosomes appear as the rod-like bodies shown in Fig. 83. In polar views 25 chromosomes can be distinguished, but the sex-chromosome cannot yet be singled out. Figs. 84 and 85 depict spermatogonial polar views. Figs. 86 to 90 inclusive picture various stages of the last spermatogonial division. Fig. 91 shows how the chromatin forms masses, each of which in turn makes a leptotene thread.

No detailed study of the growth period was made and consequently the nature of synapsis cannot be set forth. However, in the aceto-carmine smears made for hasty observations, the spireme was much plainer than in the sectioned material and in several cells, all the loops could be followed proving that the spireme was more or less continuous. In one cell in particular the spireme was composed of two parallel threads which were looped and twisted side by side. Figs. 92, 93, and 94 are reproductions from the aceto-carmine smears. Fig. 95 is a drawing

from fresh material in which the loops of the spireme could be made out. Quadripartite bodies similar to those of *Sympetrum* are formed from segments of the spireme. At first the longitudinal arms of these quadripartite crosses are longer than the transverse arms; but after the formation of the spindle, the four arms are nearly equal in length and the central part is less dense than the arms.

In a polar view of the primary spermatocyte division, 12 chromosomes can always be counted. These are arranged in an irregular ring of 8, surrounding four central chromosomes. More than 200 polar views were observed in which this number was present. Twelve was, therefore, thought to be the reduced number until the behavior of the chromosomes in this division was ascertained. It was then discovered that one chromosome, presumably the sex-chromosome, in nearly all cells goes over in advance of the autosomes to one pole. In consequence of such behavior, a polar view in which this chromosome is visible in the metaphase is difficult to find. Fig. 96, however, shows 13 chromosomes in a polar view and this is undoubtedly the correct number for the primary spermatocyte. Fig. 97, taken from a smear, shows only 12 chromosomes. Figs. 98 and 99 are drawn from the same cell at two different focal levels and in 99 the sex-chromosome appears above the autosomes. Figs. 100 to 103 inclusive, represent side-views of spindles with the sex-chromosome in advance of the autosomes. Fig. 104 is from an aceto-carmine smear and does not show the sex-chromosome. Figs. 105 to 109 are of especial value as they are drawn from living unstained tissue teased out in salt solution. The chromosomes could be distinguished from the protoplasm and the spindle fibers by the way in which they refracted the light. All stages in division could be found and one cell was watched while it underwent the change from the metaphase to the telophase stage, where further division stopped due probably to the fact that the solution was not absolutely isotonic with the cell protoplasm. Figs. 110, 111, and 112 are telophase stages of this division. Fig. 113 is the same stage drawn from a smear preparation.

In the secondary spermatogonial division two classes of cells

are present; one containing 12 chromosomes and the other 13 chromosomes. Figs. 114, 115, 116, are polar views of secondary spermatocytes. Fig. 116 shows two cells formed from one primary spermatocyte lying side by side, and in one there are 13 chromosomes and in the other 12. In one cell the sex-chromosome seemed to pass undivided to one pole as shown in Fig. 117 and in Fig. 118 it remains undivided at the metaphase. Figs. 119 and 120 represent secondary spermatocyte spindles while in Figs. 121 and 122 the dividing sex-chromosome stands out distinctly from the autosomes. Fig. 123 is a telophase stage.

After the second maturation division the chromosomes become fused into several large masses connected by a reticulum as in *Sympetrum*. The irregularity of the grouping leads to the conclusion that the number of masses has nothing to do with the presence or absence of the sex-chromosome and the difference in the amount of chromatin is not noticeable in the spermatids. The cytoplasm around the nucleus elongates and the centrosome is found near the largest mass of chromatin. The axial filament grows out from the centrosome which increases in size until it forms a knob. This is the only thing comparable to a middle-piece, and as the nucleus elongates it becomes so closely associated with the large chromatin mass that it can no longer be distinguished from it. Figs. 124 to 128 inclusive are drawn from living material. The spermatozoön moved with a peculiarly spiral motion such as that described for *Sympetrum*. In Fig. 127 the cytoplasm is spread out at the base of the head, probably an abnormal condition. The spermatozoön did not move rapidly but progressed continuously. In the mature spermatozoön no head-spine was visible upon the rod-like head which comprises about one third of the length of the whole spermatozoön.

VI. Historical Review.

There has been little work done upon the cytology of the Odonata. Of historical interest only are the papers by C. Th. von Siebold (1840) and Bütschli in 1871. Von Siebold deals mostly with the mating habits of the Libellulidæ, though he made some observations upon the spermatozoa. According to his account, the spermatozoa have in general the characteristic

elongated form of the insect spermatozoa and may be divided into two classes:

1. In the genera *Agrion*, *Æschna* and *Diastatomma*, they are fine, capilliform and extremely motile.

2. In the genus *Libellula* he affirms they are more solid and rod-like, and remain inactive in the male and even in the female after fertilization.

In all genera, the spermatozoa develop in the testis in bundles surrounded by delicate sheaths. In *Æschna ocellata* these bundles are so large that they can be recognized with the naked eye as white dots in the testis. In group (I) the bundles are round or oval and somewhat compressed. Before the maturation of the spermatozoa in the cyst surrounded by a sheath, there is a big vesicular area which enlarges and becomes finely granular. This is the beginning of the formation of the spermatozoa which arise within the bladder-like area of the cyst.

Von Siebold was uncertain as to the immobility of the spermatozoa in the Libellula for he says, "Ob diese Spermatozoen unter gewissen Bedingungen, welche mir entgangen sind, sich nicht dennoch bewegen solten, weiss ich nicht zu sagen."

Bütschli worked out a number of the developmental stages of the head of the spermatozoön of *Agrion puella*. He described the nucleus as sending forth a small elongation which increases in length and forms an opaque head-spine. In an immature spermatozoön, it measured 0.01 to 0.045 mm. in length while in the ripe spermatozoön it was reduced to .0078 to .009 mm. He thought that there must be some deception in his results, but in *Sympetrum* the same thing occurs.

Lefevre and McGill (1908) extended and corrected the earlier paper (1904) of McGill on *Anax junius*. The spermatogonial number was found to be twenty-seven. The small or m chromosomes of the spermatogonium divided at both mitoses and was distinct from the accessory which was a larger chromosome in the spermatogonial group. A condensed chromosome-like body persisting through the various growth stages was identified as the odd or heterotropic chromosome of the maturation division. They also found evidence which suggested that the synapsis might be a side to side union of the threads instead of end to end as first described.

In the formation of the tetrad, they found that the long axis of the tetrad is identical with the long axis of the chromatin threads of the growth period, and the first maturation division would separate univalent chromosomes and be a reducing division if the conjugation took place end to end. To quote the rest of the conclusion, "If, however, it should prove true of this form that a parallel conjugation occurs, as has been suggested, the first division would still be a reducing one, since the axis of the crosses are not reversed by the drawing out of the transverse arms and the attachment to the spindle fibers is at the end of the longitudinal arms."

Zeleny and Faust ('15) in the figures of Lefevre and McGill ('08) have measured the chromosomes and calculated a bimodal curve for the two classes of spermatozoa which must result from the behavior of the odd chromosome. "These give expected ratios of 1.00 : 1.07 on the basis of complete fusion of chromosomes and production of spermatozoa of like shape and 1.00 : 1.09 for end to end fusion of chromosomes." The spermatozoa of *Æschna canadensis* in which the chromosomal content is unknown give a bimodal but unequal curve. "The two modes are at 50.2 μ and 51.6 μ, giving a ratio of 1.00 : 1.03. This is considerably less than the ratio 1.00 : 1.07 or 1.00 : 1.09 that would be expected for *Anax junius*."

VII. General Considerations.

In *Anax*, *Sympetrum* and *Libellula*, the spermatogonial chromosomes are 27, 25, and 25 respectively. Throughout the growth period, a condensed chromatin body persists which from its subsequent behavior can be identified as the sex-chromosome. In the spermatocyte, a synapsis occurs and the autosomes form quadripartite bodies, all of which are bivalent. In the first division in *Anax* and *Sympetrum* the bivalents and the sex-chromosomes divide, and this division as far as the former are concerned probably represents a pulling apart of the univalent chromosomes which conjugated in synapsis and is therefore a true reduction division. Upon this assumption, the second division which splits the univalents must be equational. The sex-chromosome undivided goes over to one pole in the second

division. In *Libellula*, upon assuming parasynapsis, the first division represents a true reduction in every way for the bivalents divide and the sex-chromosome goes to one pole undivided. Certain stages enumerated in the description of *Sympetrum* differ from what Lefevre and McGill described for *Anax*.

VIII. Summary.

1. The maturing sex-cells are arranged in cysts in the testes, but there is no definite seriation as to age like that found in many insects and vertebrates.

2. The spermatogonial chromosomes are twenty-five in number and are closely crowded together, making it impossible to tell much about their behavior.

3. The evidence obtained seems to indicate that the leptotene threads unite side by side (parasynapsis) to form a spireme which is twisted in such a way that the loops are oriented toward one side of the nucleus.

4. This spireme breaks up into segments which open out presumably along the original axis of synapsis to form rings. These condense into crosses and then into quadripartite bodies or prophase chromosomes.

5. The primary spermatocyte contains 12 bivalent autosomes and one sex-chromosome. The bivalents divide apparently along the line of their original junction making this the reduction division for them while the sex-chromosome divides equationally.

6. In the second spermatocyte division all the univalent autosomes divide equally while the sex-chromosome passes to one pole undivided; thus two kinds of spermatids are formed. These change into linear spermatozoa which show no visible difference. But the one which possesses the sex chromosome must be physiologically different.

7. In *Libellula basalis* the spermatogonial number is 25; the reduced number, 13, consists of 12 bivalent autosomes and 1 univalent sex-chromosome. The sex-chromosome, unlike its procedure in *Sympetrum*, passes undivided to one pole in the primary spermatocyte division, forming two kinds of secondary spermatocytes. In the secondary division the sex-chromosome divides equally. Two kinds of spermatozoa are thus formed which must have a functional difference.

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EXPLANATION OF PLATES.

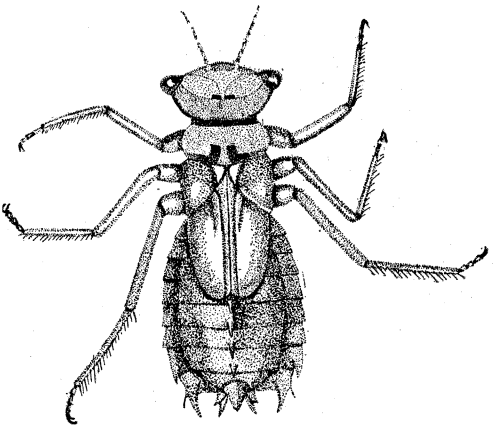
Unless otherwise indicated the figures are drawn at a magnification of 2,700 diameters. The drawings of the fresh material are made with a magnification of 1,500 diameters. Fig. 24 is magnified 3,900 diameters. Plate I. is reduced one-third while Plates II. to VI. inclusive are reduced one-fifth. The nuclei drawn are usually from sections not directly through the center of the cells, as they contain less chromatin and are consequently clearer.

PLATE I.

Sympetrum semicinctum (Say).

FIG. 1. A drawing of the nymph of *Sympetrum semicinctum* (Say).

FIG. 2. A cross-section of the testis of a nymph.



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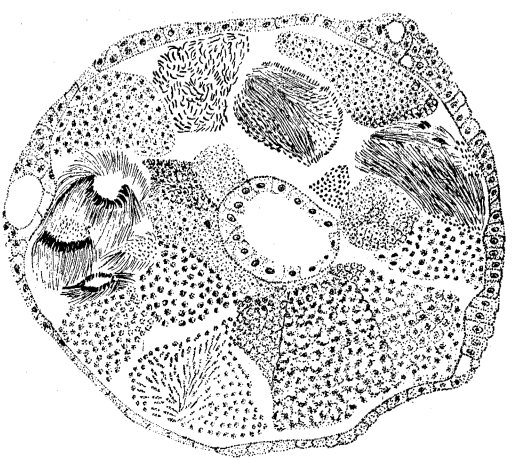

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PLATE II.

Sympetrum semicinctum (Say).

FIG. 3. Part of a cyst showing degenerating spermatogonia.

FIG. 4. A group of spermatogonial cells.

FIG. 5. A spermatogonial cell from a smear preparation.

FIG. 6. Polar view of spermatogonial chromosomes from an aceto-carmine smear.

FIG. 7. Polar view of metaphase of spermatogonial nucleus in which only a few chromosomes are in focus.

FIG. 8. Polar view of metaphase of spermatogonial nucleus.

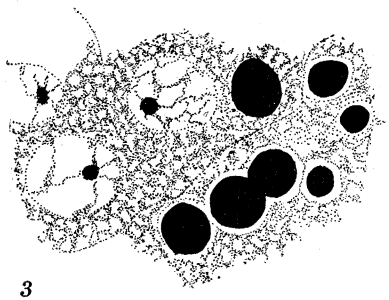
FIGS. 9, 10, AND 11. Telophases of spermatogonial divisions.

FIGS. 12 AND 13. Polar views of telophases of last spermatogonial division.

FIGS. 14, 15, AND 16. Spermatogonia with the round, dense body that may correspond to the mass in the growth period which subsequently becomes the sex-chromosome.

FIG. 17. Spermatogonia in the diffuse period before the growth changes begin.

FIGS. 18, 19, AND 20. Massive bodies in the nuclei at the beginning of growth period. One mass which later forms the sex-chromosome is much darker and more compact and is marked *X*.



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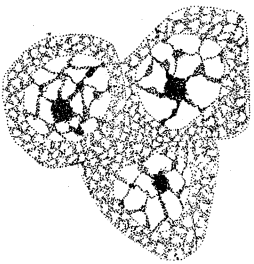

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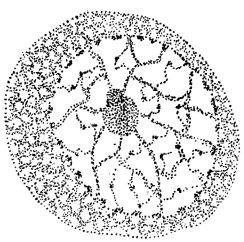

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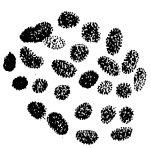

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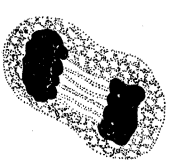

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PLATE III.

Sympterum semicinctum (Say).

FIGS. 21, 22 AND 23. Leptotene threads forming from the masses. In 22 and 23 the sex-chromosome is present.

FIGS. 24 (× 3,900), 25 AND 26. Synaptic stages in which parallel leptotene threads unite.

FIGS. 27, 28 AND 29. Synizesis stages.

FIGS. 30 AND 31. Polar views of cut threads of a stage like Fig. 29.

FIGS. 32 AND 33. Chiasmas.

FIG. 34. Threads opened out into apparent eights.

FIG. 35. A true eight.

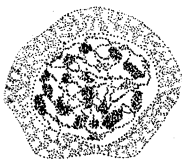
FIG. 36. A cell of *Anax junius* which shows chromomeres in the segments.

FIG. 37. Cell with signet-ring loop.

FIG. 38. Signet-ring loop from several angles.

FIGS. 39, 40 AND 41. Contain prophase crosses. Fig. 40 shows the sex-chromosome and Fig. 41 contains the largest cross.

FIGS. 42, 43 AND 44. Polar views of the chromosomes of the primary spermatocyte division.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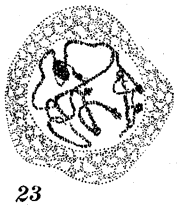
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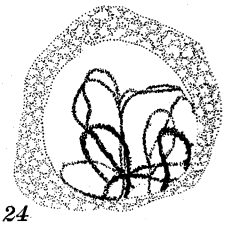
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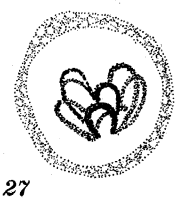
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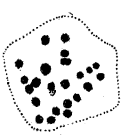
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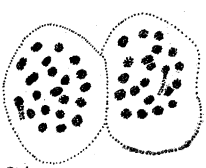
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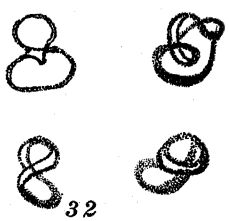
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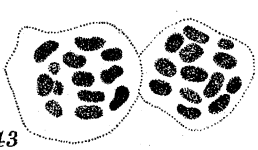
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PLATE IV.

Sympetrum semicinctum (Say).

FIGS. 45, 46, AND 47. Side views of metaphase of primary spermatocyte division.

FIG. 48. Anaphase of primary spermatocyte division.

FIGS. 49, 50, 51 AND 52. Telophases of primary division.

FIG. 53. Polar view of telophase of primary division which shows the sex-chromosome at one side.

FIGS. 54, 55, 56 AND 57. Polar view of chromosomes of secondary spermatocyte.

FIGS. 58, 59, 60, 61 AND 62. Metaphase views of secondary spermatocyte divisions with the sex-chromosome in various positions.

FIGS. 63, 64. Telophases of secondary spermatocyte division.

FIG. 65. Metaphase of secondary spermatocyte division from aceto-carmine smear.

FIGS. 66 AND 67. Polar view of telophase of secondary spermatocyte division showing sex-chromosome.

FIGS. 68, 69 AND 70. Spermatids.

FIG. 71. Spermatid showing tail filament.

FIGS. 72 AND 73. Spermatids which have elongated and formed head spines.

FIG. 74. A sperm head from a smear preparation.

FIGS. 75 AND 76. Cross-section of spermatozoa to show the chromatin condensed around the nuclear wall.

FIGS. 77, 78 AND 79. Spermatozoa from aceto-carmine preparations.

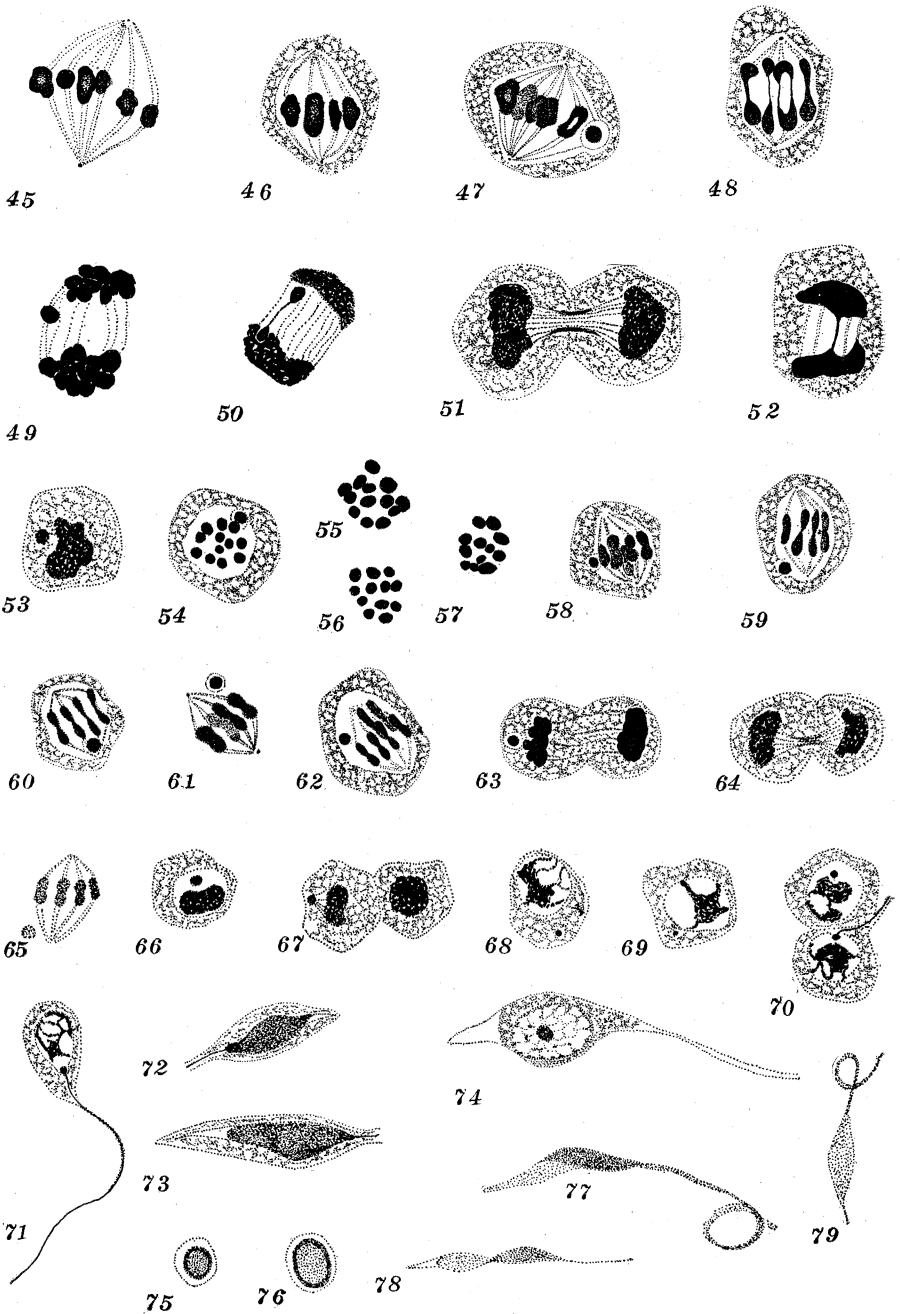

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PLATE V.

Libellula basalis.

FIG. 80. Spermatogonium from aceto-carmine preparation.

FIG. 81. Spermatogonium from iron-hematoxylin section.

FIG. 82. Spermatogonium from fresh material (× 1,500).

FIG. 83. Prophase chromosomes of spermatogonium.

FIGS. 84 AND 85. Polar view of chromosomes of last spermatogonial division.

FIGS. 86–90. Spermatogonial division stages.

FIG. 91. Formation of leptotene threads.

FIGS. 92, 93 AND 94. Spireme stages from aceto-carmine preparations.

FIG. 95. Spireme stage from fresh material.

FIGS. 96, 97, 98 AND 99. Polar views of chromosomes of primary spermatocyte division. Fig. 97 is from an aceto-carmine preparation and shows only 12 chromosomes. Figs. 98 and 99 are taken from the same cell, at different focal levels.

FIGS. 100, 101, 102 AND 103. Various stages in primary spermatocyte division showing the sex-chromosome going to one pole undivided.

FIG. 104. Metaphase of primary spermatocyte division from an aceto-carmine preparation.

FIGS. 105 AND 106. Primary spermatocyte stages (× 1,500) from fresh material.

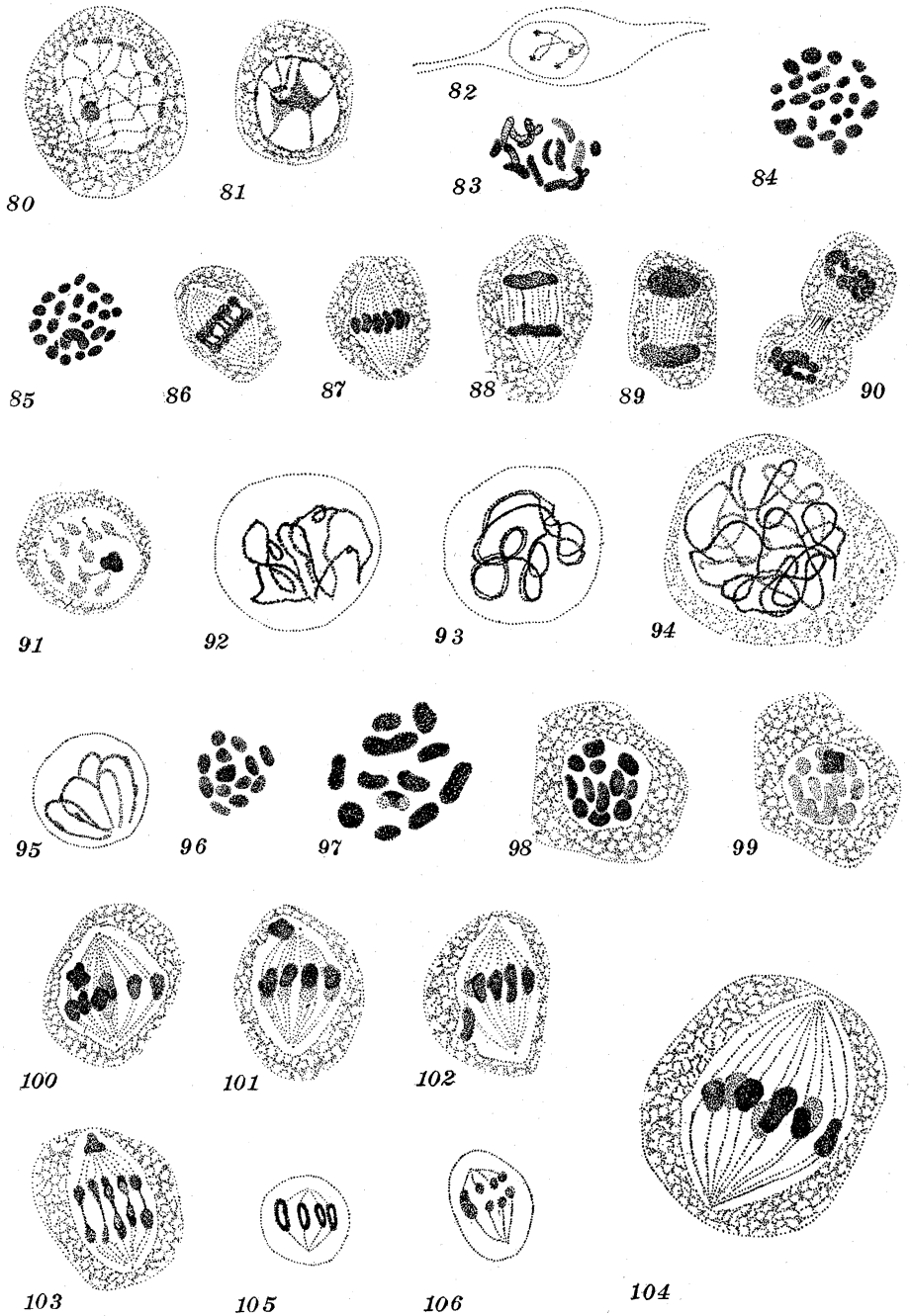

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PLATE VI.

Libellula basalis.

FIGS. 107, 108 AND 109. Primary spermatocyte stages (× 1,500) from fresh material.

FIGS. 110, 111, AND 112. Telophases of primary spermatocyte division.

FIG. 113. Telophase of primary spermatocyte division from aceto-carmine smear.

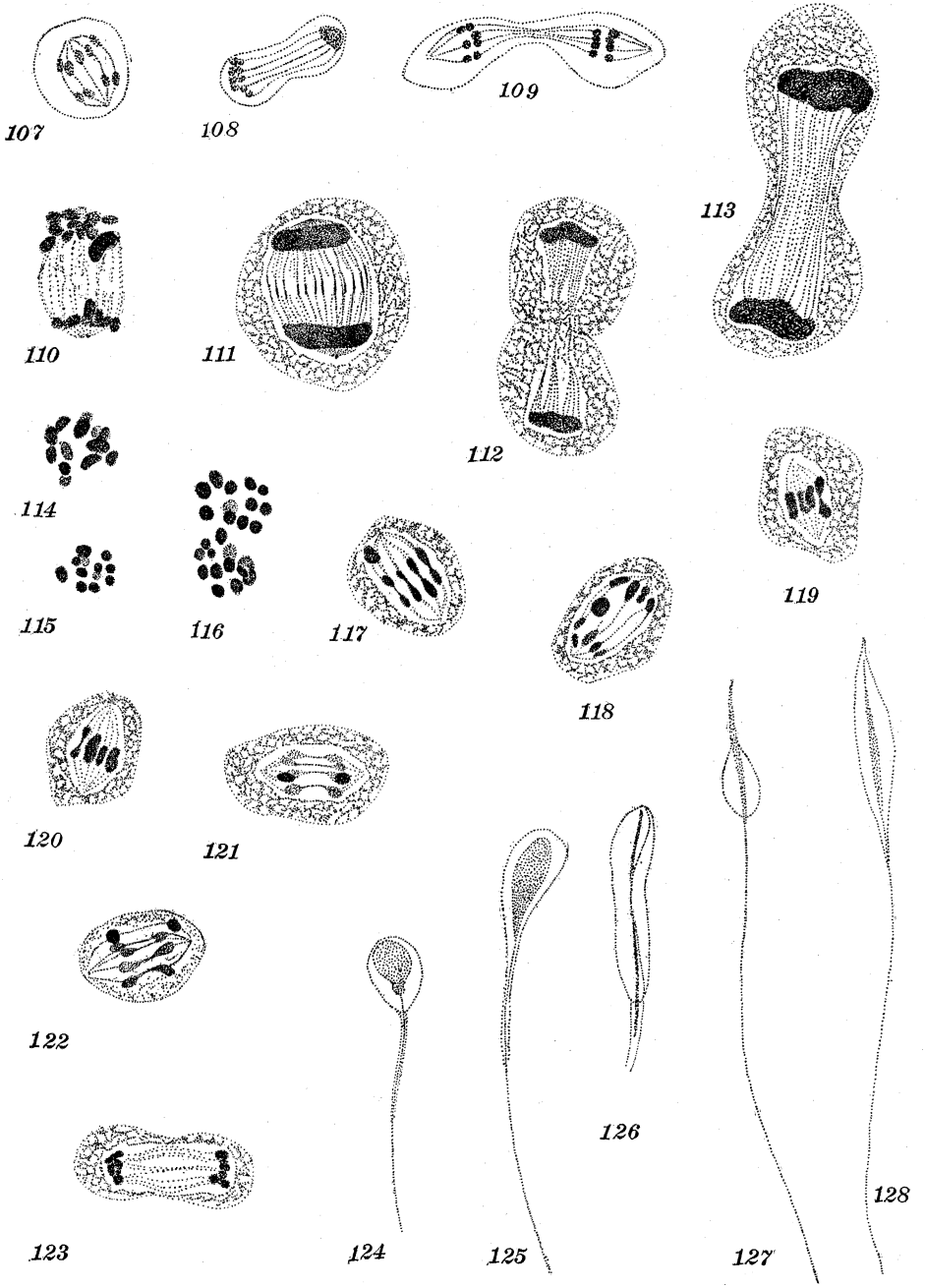
FIGS. 114, 115, AND 116. Polar views of chromosomes of secondary spermatocytes.

FIG. 117. One chromosome apparently going to one pole undivided in secondary spermatocyte division.

FIG. 118. Sex-chromosome lagging behind in secondary spermatocyte division.

FIGS. 119, 120, 121, 122, AND 123. Various stages in secondary spermatocyte division.

FIGS. 124–128 INCLUSIVE. Spermatozoa drawn from living material.



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